2011 q-bio Summer School:

# Stochastic Gene Regulation

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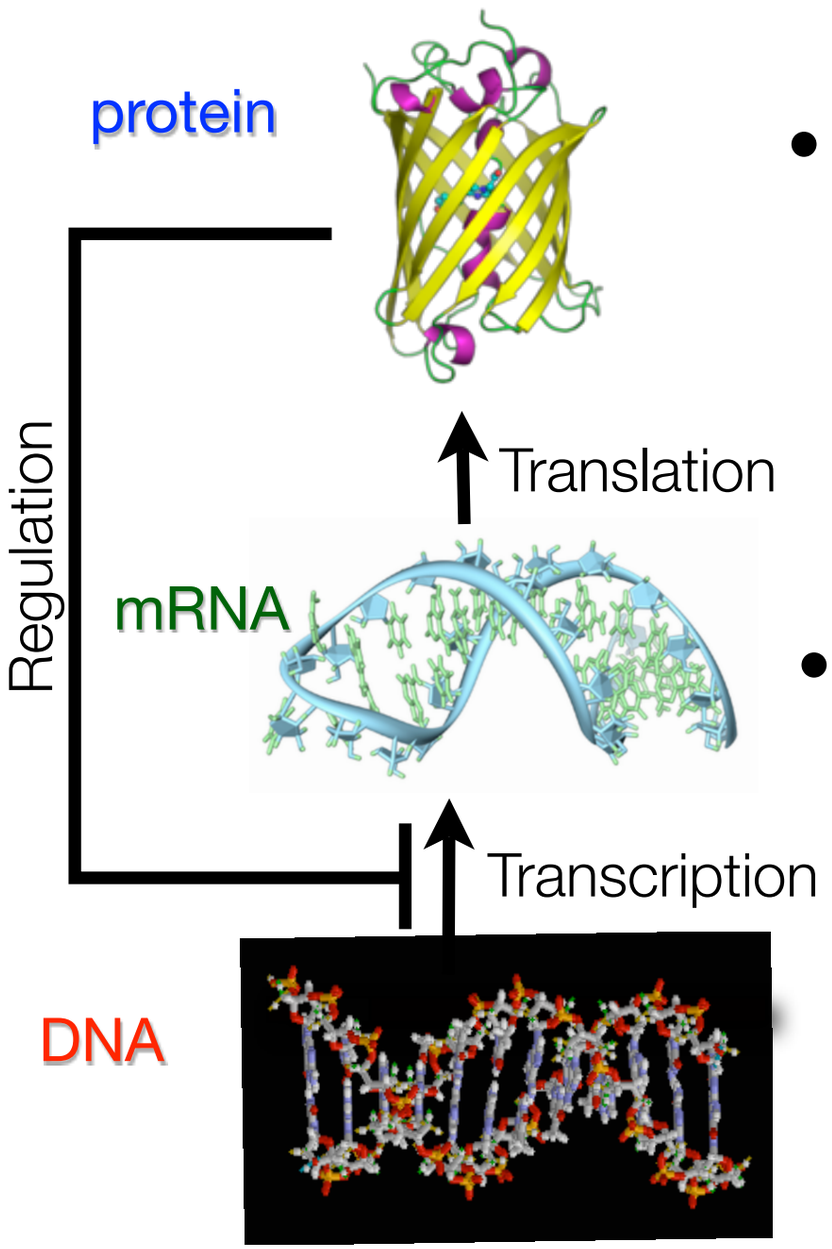
# Stochastic Biochemistry: Theme Overview

1. Stochastic Phenomena: origins and consequences.

2. Single Cell Research.

# Origins of Stochasticity:
# 1) Small molecular copy numbers



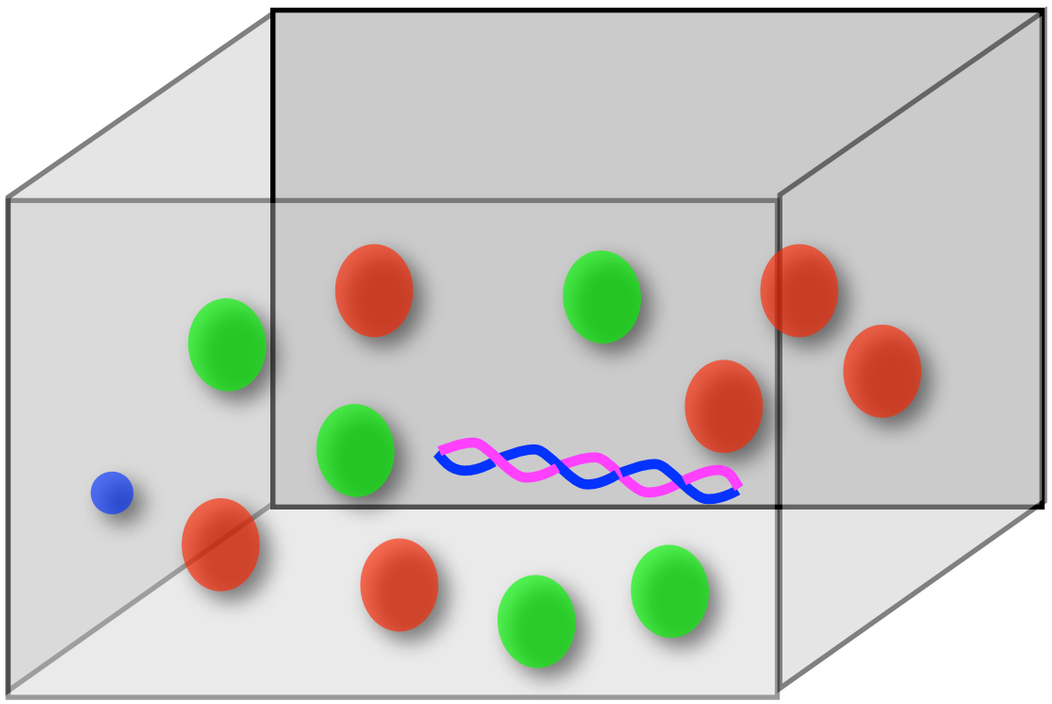

- Proteins build cellular structures, pass cellular information and regulate cellular activities. Variable copy numbers (~0-100,000/cell).

- mRNA transfer instructions for creating specific proteins. Low copy numbers (~0-100/cell).

- DNA contains all of the genetic instructions. Extremely low copy numbers (~0-5/cell).

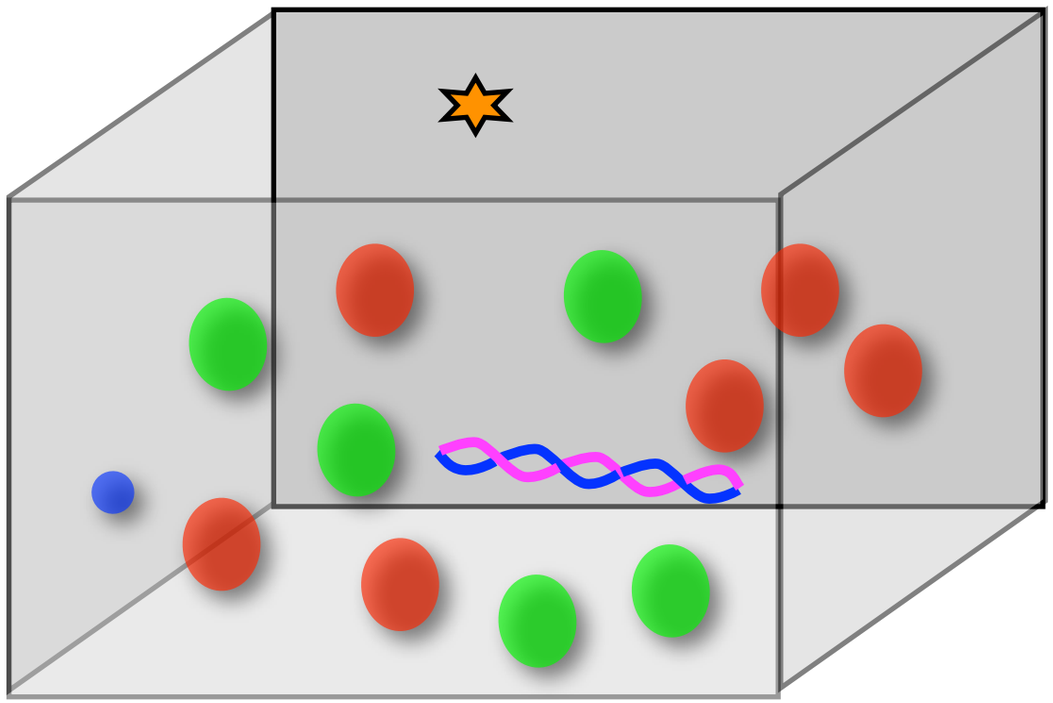
## The Central Dogma of Molecular Biology

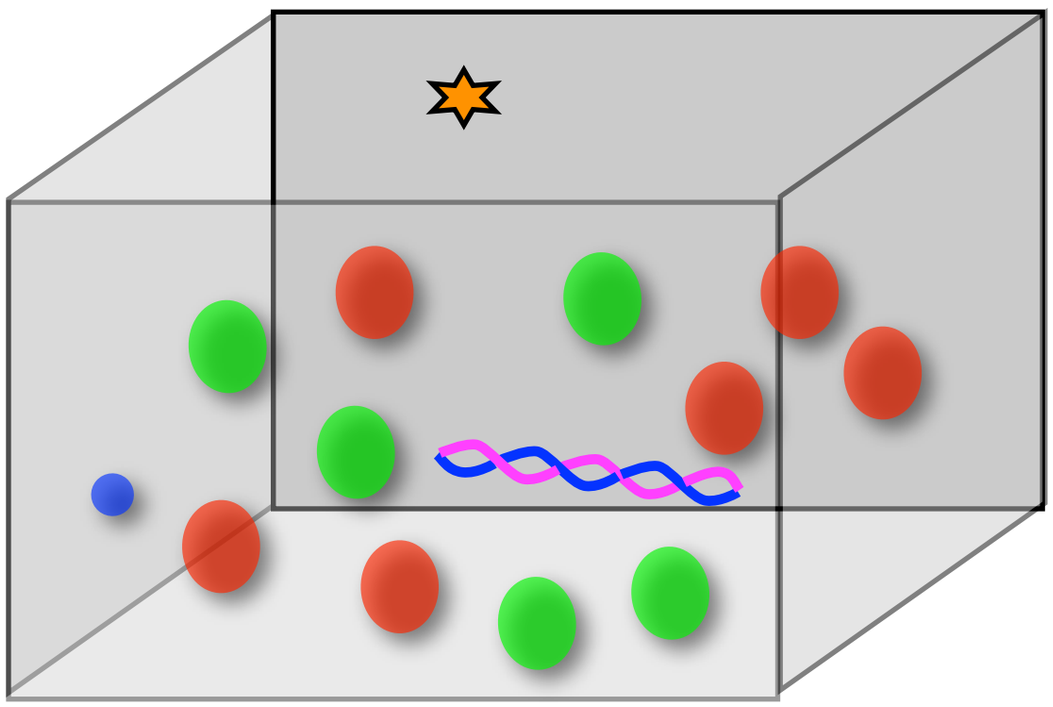
# Origins of Stochasticity:
# 2) Spatial fluctuations of cellular constituents.



Thermal fluctuations will lead to randomness in times between reactions.

# Origins of Stochasticity:
# 3) Competition of different events.





Different reactions will lead to different consequences.

Which ever molecule wins the race will define the reaction.

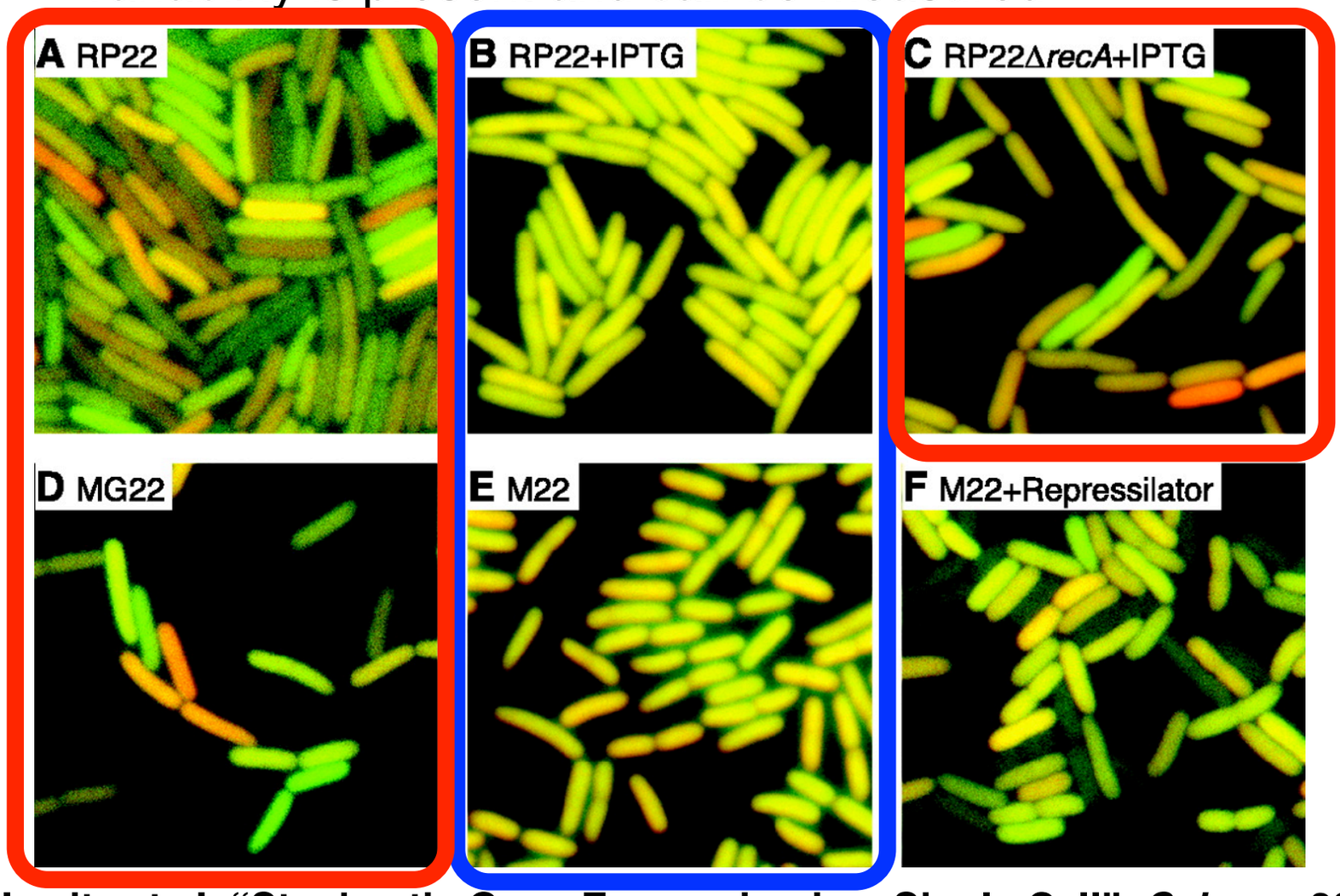
# Origins of Stochasticity:
# 4) Extrinsic fluctuations.

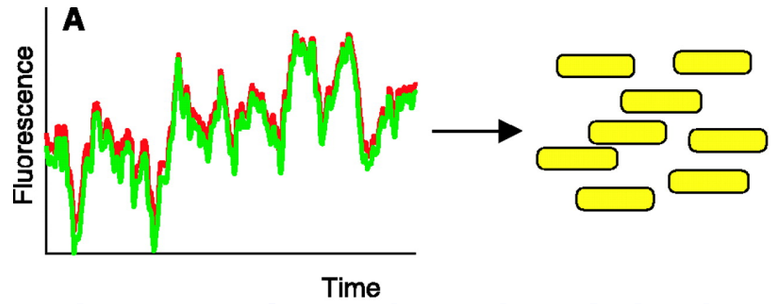
Changes in temperature, nutrients, radiation, chemicals, pressure, etc...

Fluctuations of upstream genes, intercellular signals.

# Intrinsic versus Extrinsic Noise

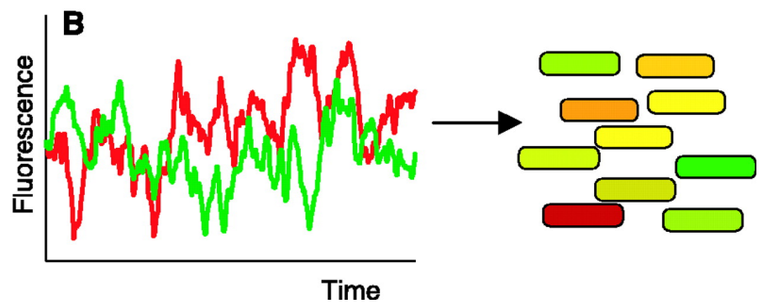
- Variability is present and can be measured




Low Intrinsic Noise

High Intrinsic Noise




**Elowitz et al, "Stochastic Gene Expression in a Single Cell", *Science* 2002**

- Inserted two reporters on the chromosome (cfp, yfp)
- Each was controlled by the same promoter
- Expression of cfp shown in green, yfp in red

# Stochastic Effects Lead to Phenotypical Differences


Fingerprints of identical twins


Cc, the first cloned cat and her genetic mother, Rainbow

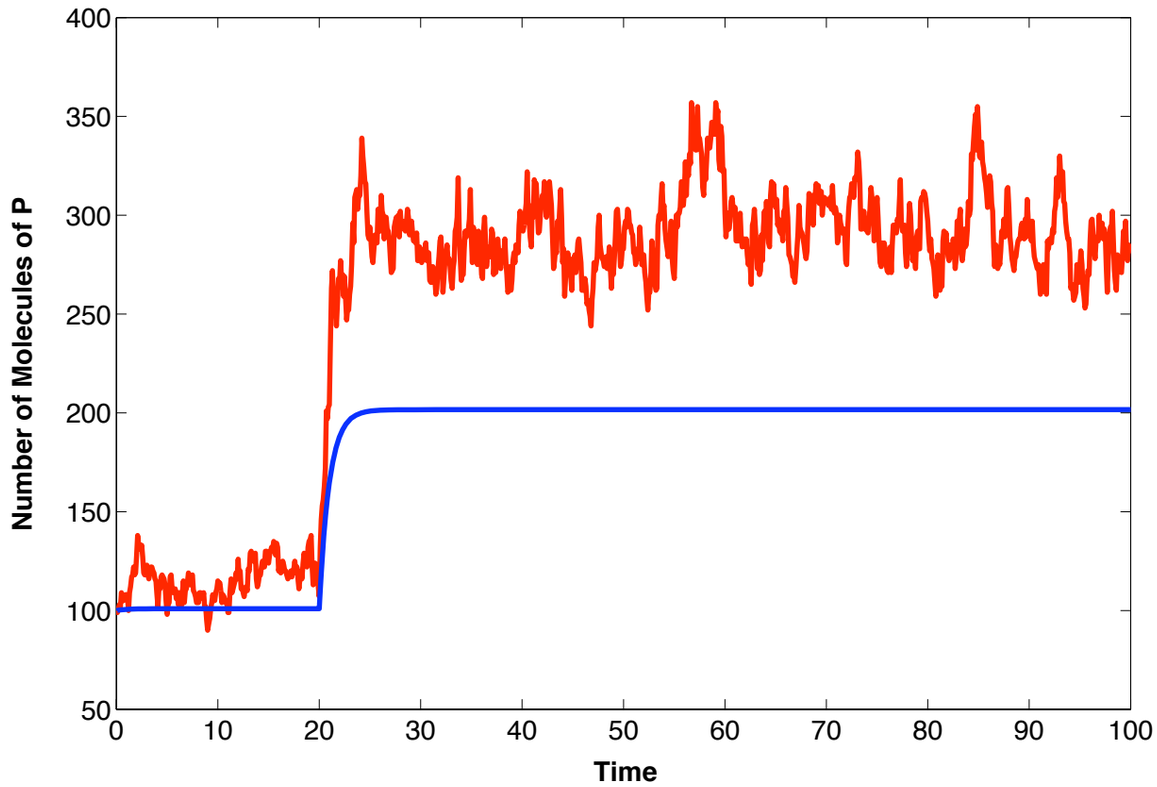
**J. Raser and E. O'Shea, "Noise in Gene Expression: Origins, Consequences, and Control", *Science*, 2005**

Slide Contributed by Mustafa Khammash

# Stochastic Phenomena:
# 1) Signal Amplification (or damping).

$$\phi \underset{k_a S}{\overset{k}{\rightleftharpoons}} I \xrightarrow{k_p} P \xrightarrow{1} \phi$$

$$\phi \underset{k_d}{\overset{k_s}{\rightleftharpoons}} S$$

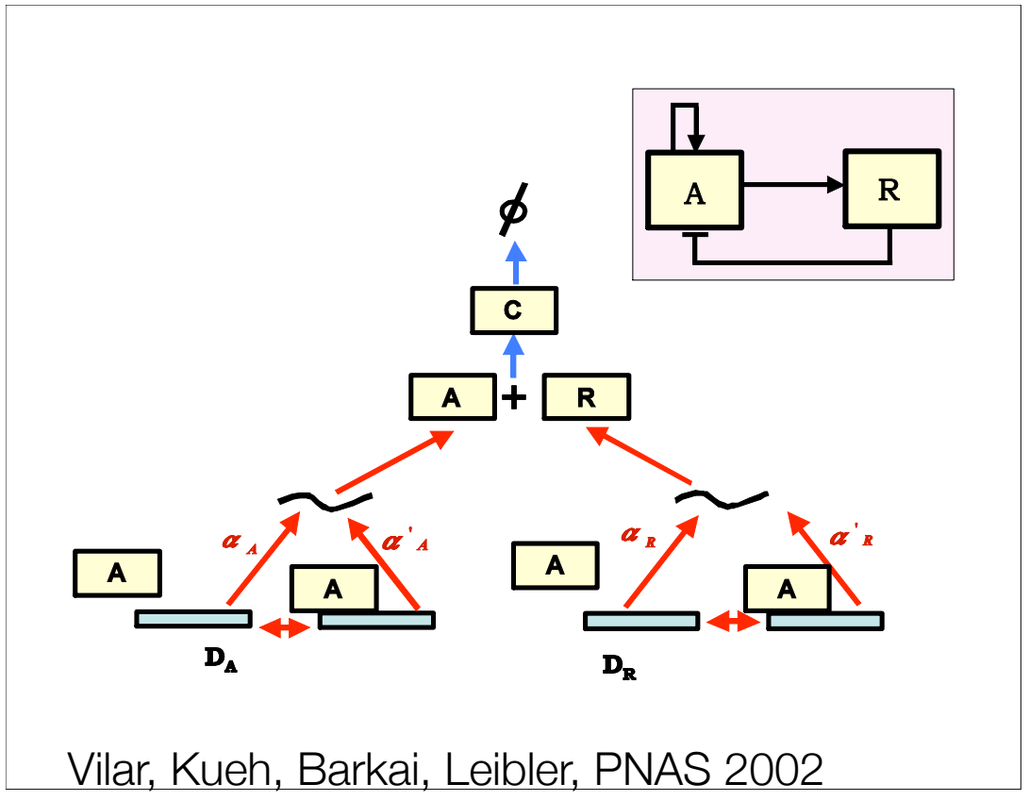



**Johan Paulsson , Otto G. Berg , and Måns Ehrenberg, "Stochastic Focusing: Fluctuation-enhansed sensitivity of intracellular regulation" PNAS 2000**

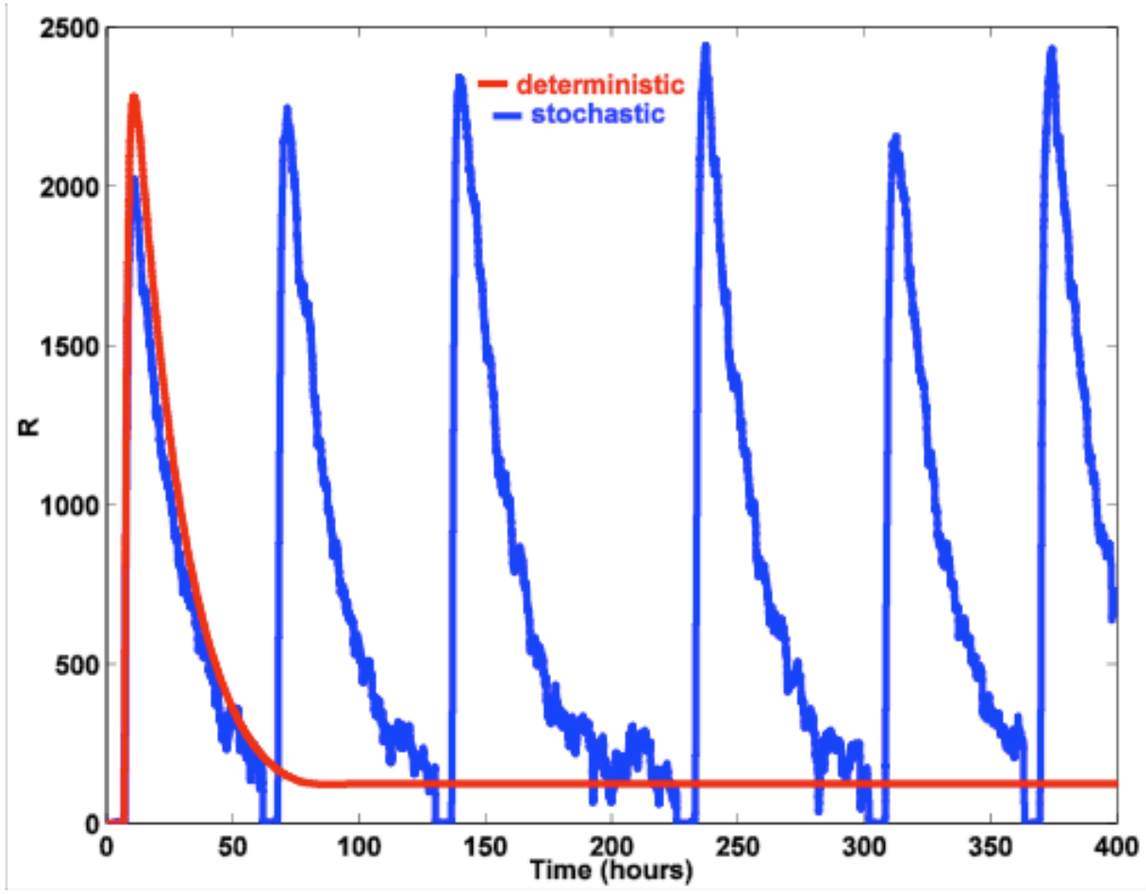
- Stochastic mean value different from deterministic steady state
- Noise *enhances* signal!

# Stochastic Phenomena:
# 2) Noise Induced Oscillations

## Circadian rhythm



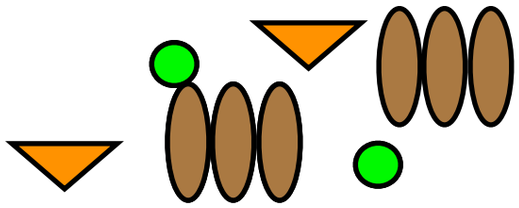

Vilar, Kueh, Barkai, Leibler, PNAS 2002




- Oscillations disappear from deterministic model after a small reduction in deg. of repressor
- (Coherence resonance) Regularity of noise induced oscillations can be manipulated by tuning the level of noise [*El-Samad, Khammash*]

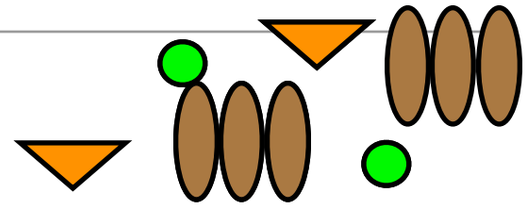
Slide Contributed by Mustafa Khammash

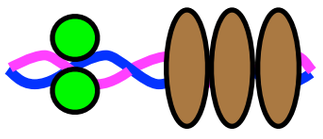
# Stochastic Phenomena:
# 3) Stochastic Switching



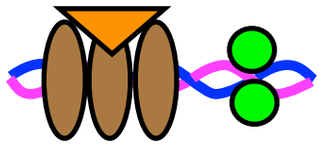
Same chemical environment.

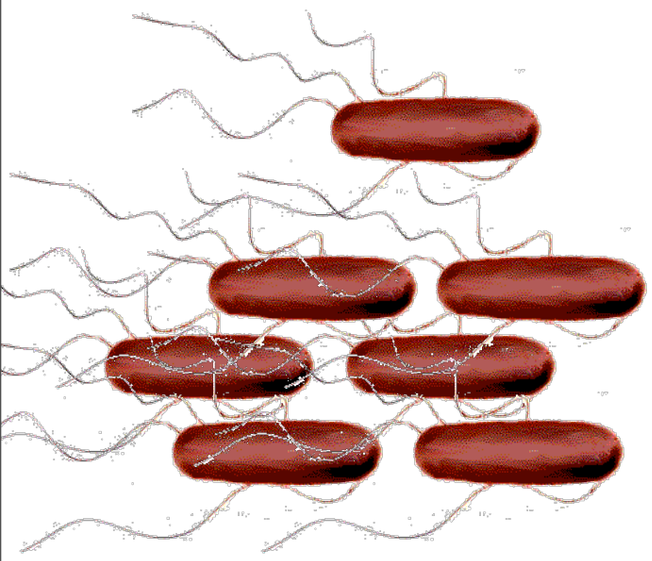
Same genetic code.



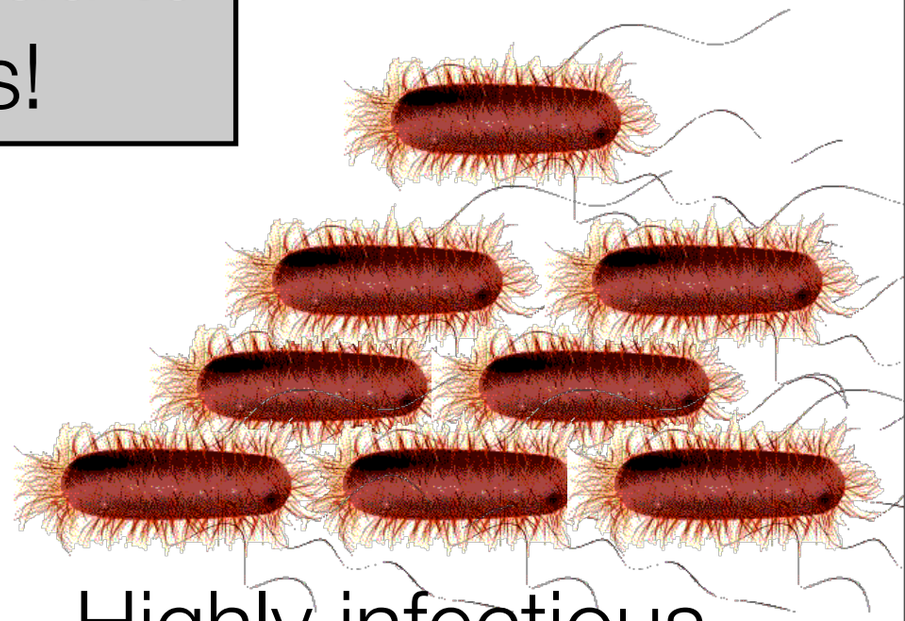


Random reactions can lead to vastly different results!





Harmless phenotype.



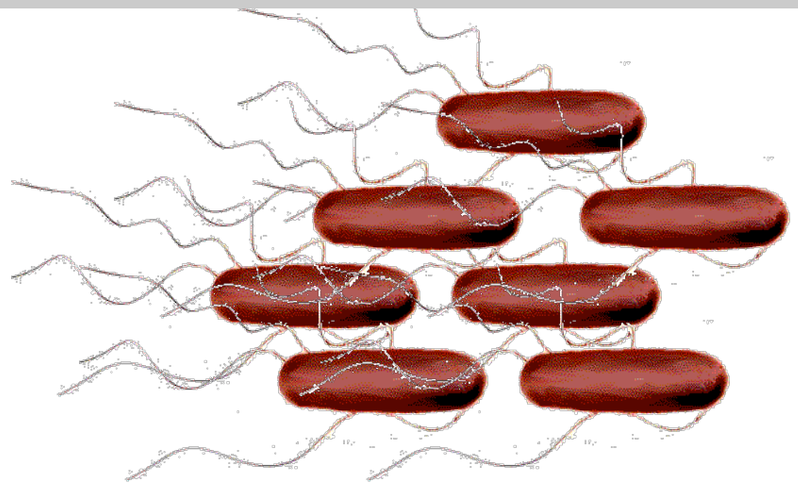
Highly infectious phenotype.

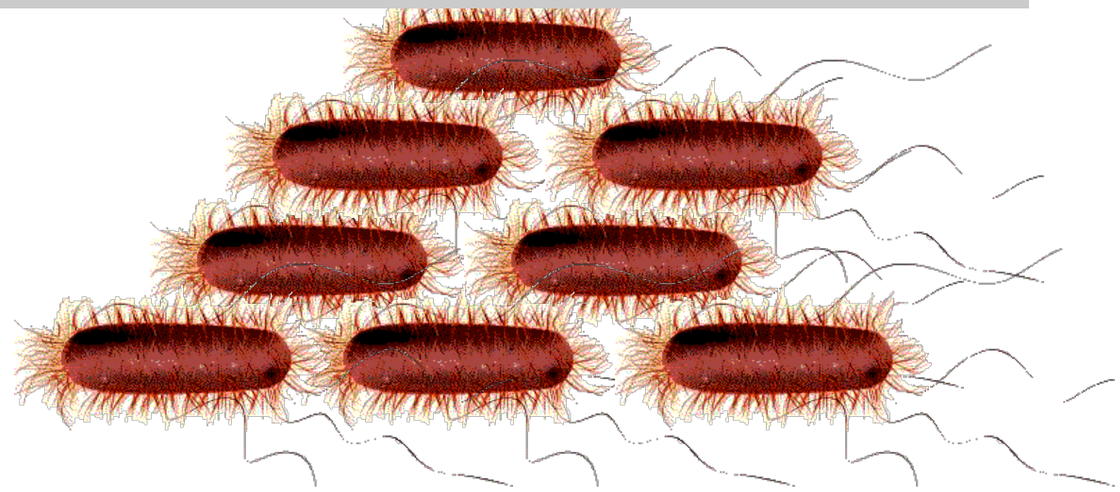
Munsky, Trinh, Hernday, Khammash, Low, *under preparation,* 2011

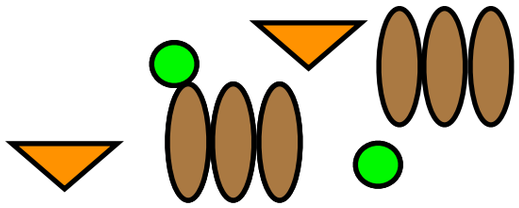
# The Importance of Single Cell Analyses

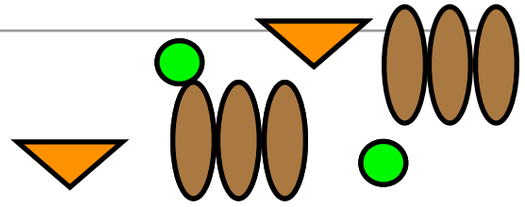
For these systems, we need
single cell analyses to answer:

★ What will happen?
★ How frequently?
★ Why does it happen?
★ Under what conditions?
★ What advantages does it provide?
★ How can we prevent it?
★ How can we cause it?









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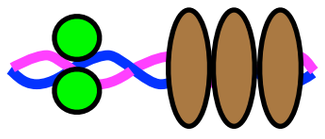

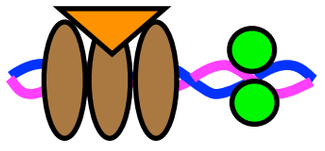

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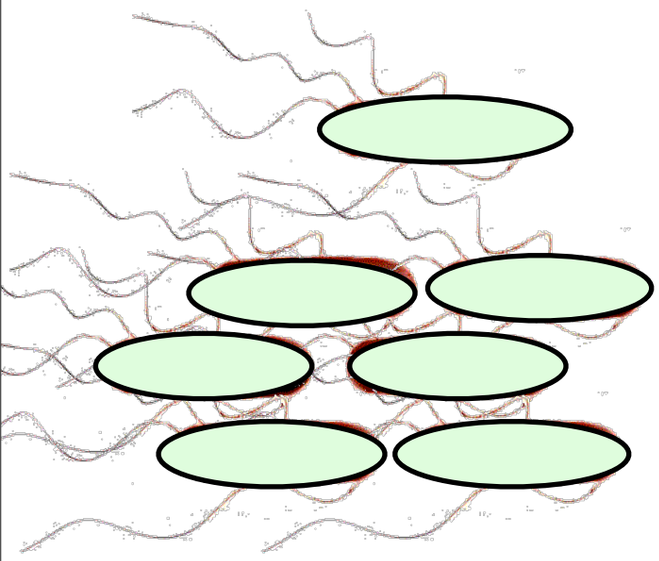
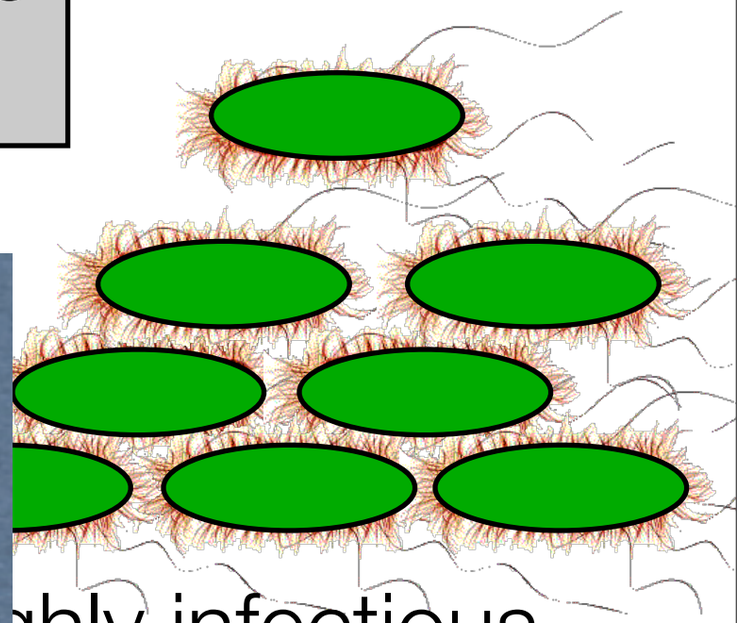


Harmless phenotype.

Genetic manipulations make it easy to see changes under the microscope.



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Munsky, Trinh, Hernday, Khammash, Low, *under preparation,* 2010

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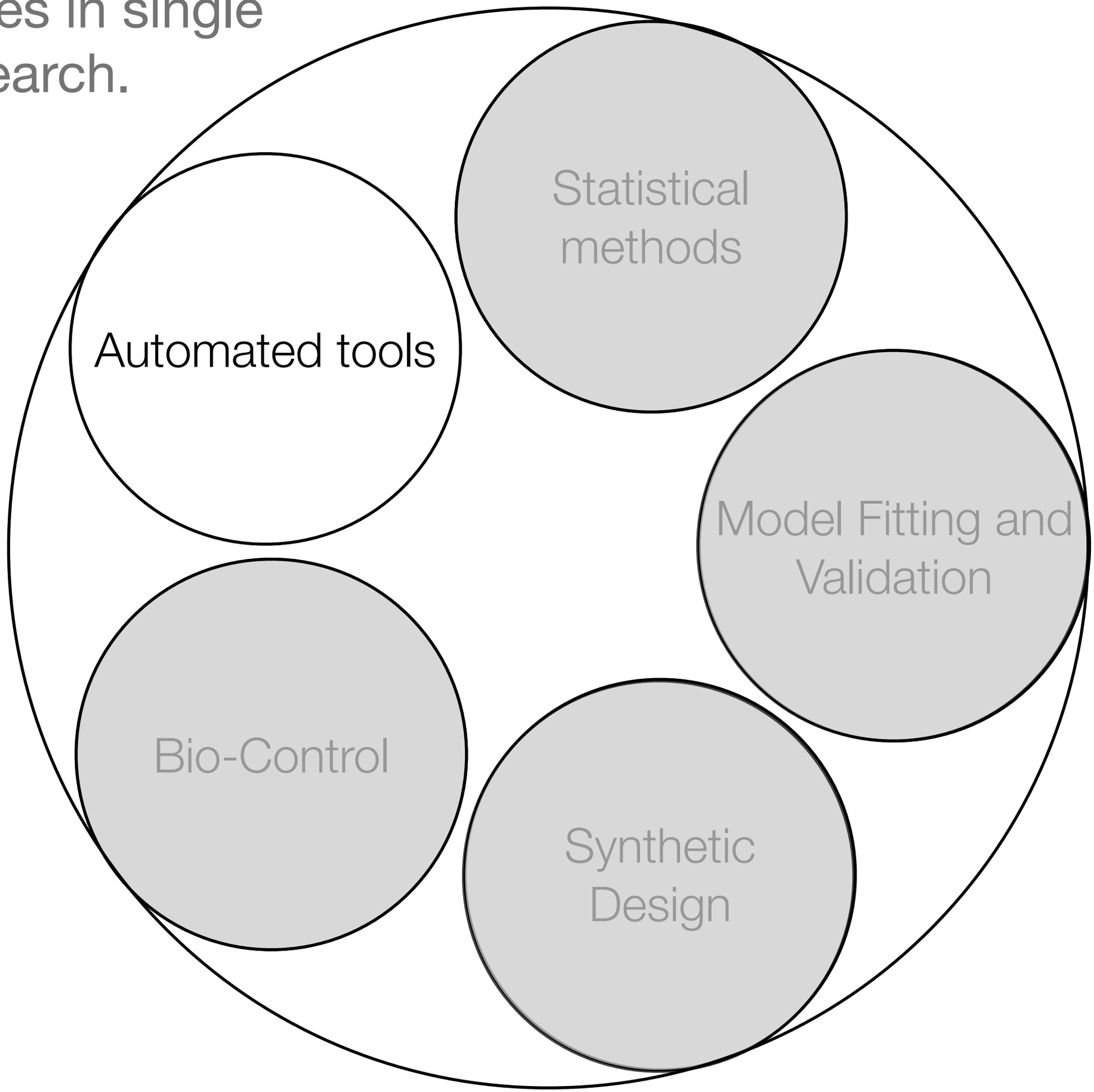
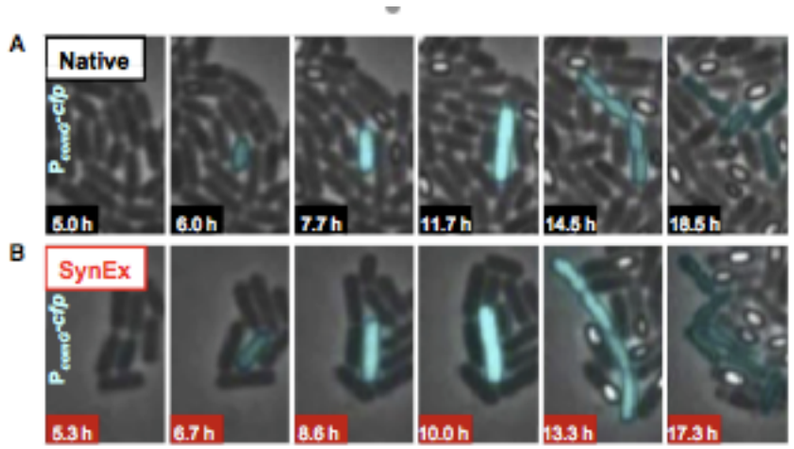
Advances in single cell research.

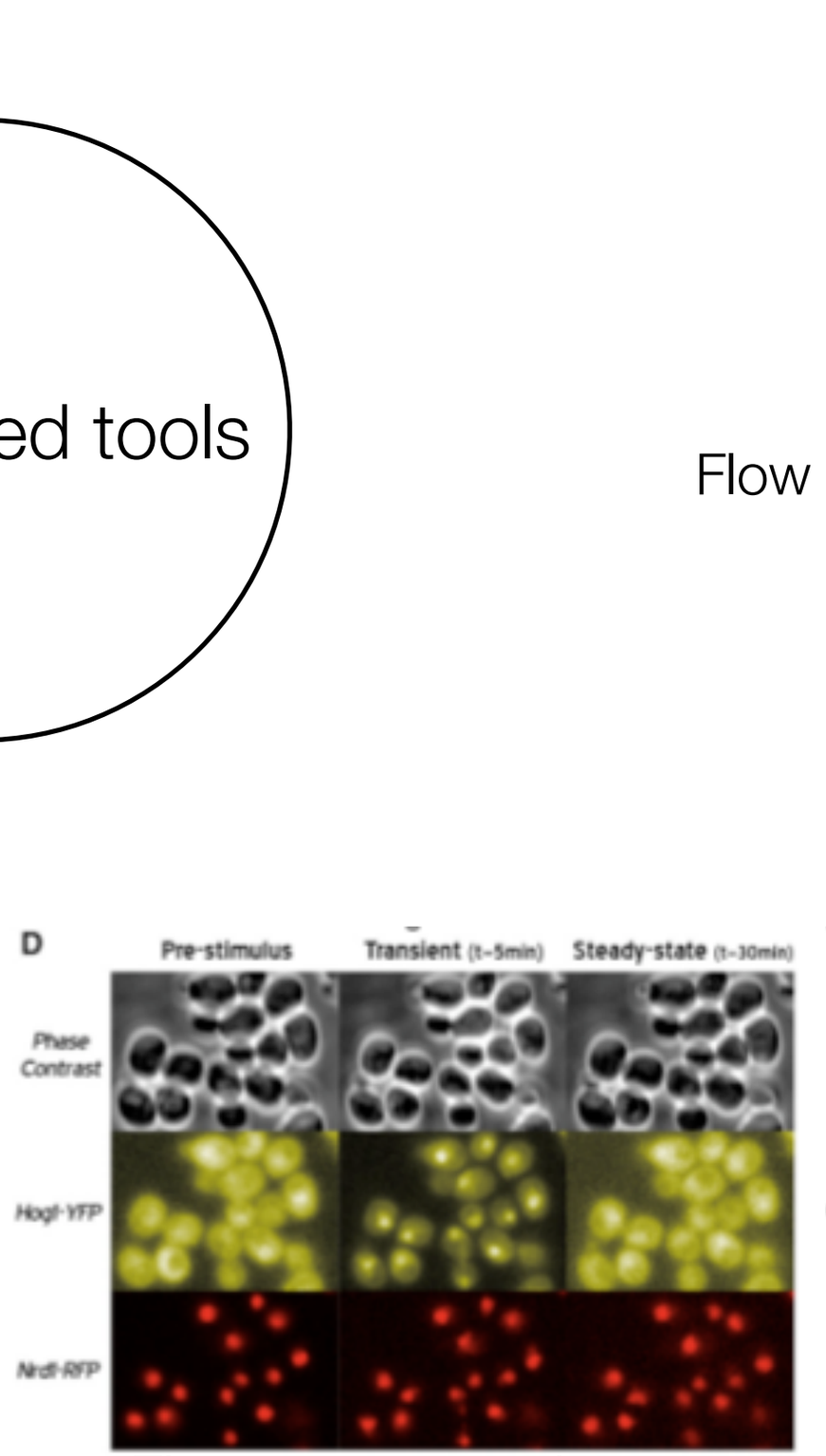


Automated tools



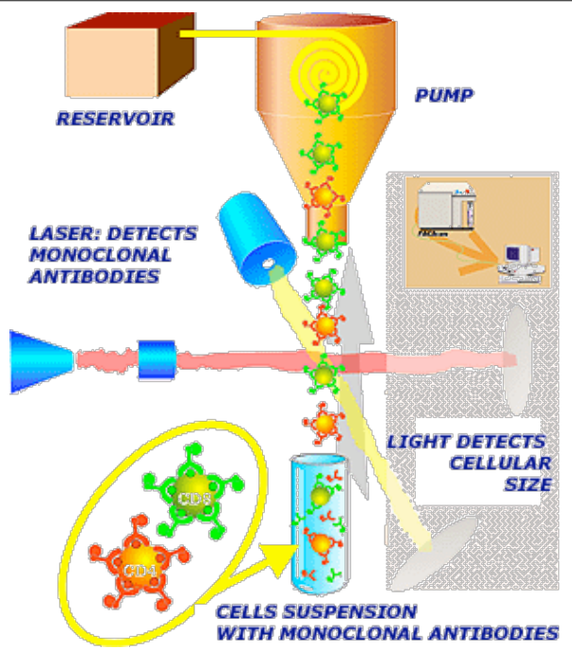

Time lapse fluorescence microscopy

**Cagatay et al, Cell 2009**

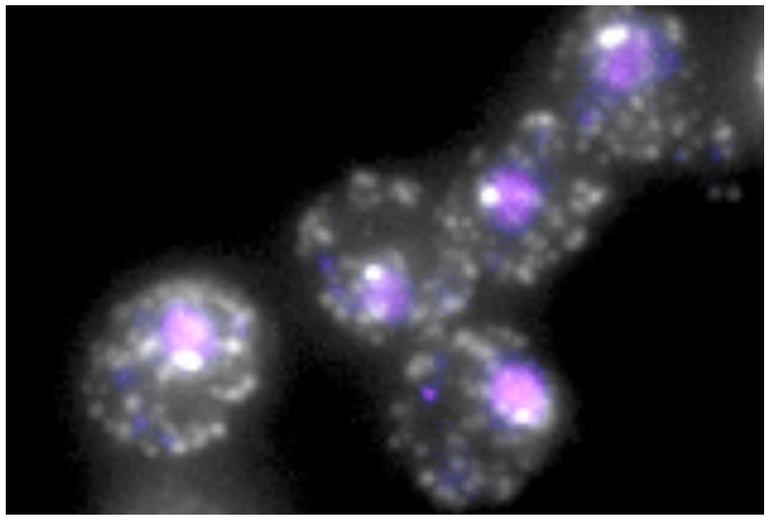



Fluorescence microscopy,

**Muzzey et al, Cell 2009**



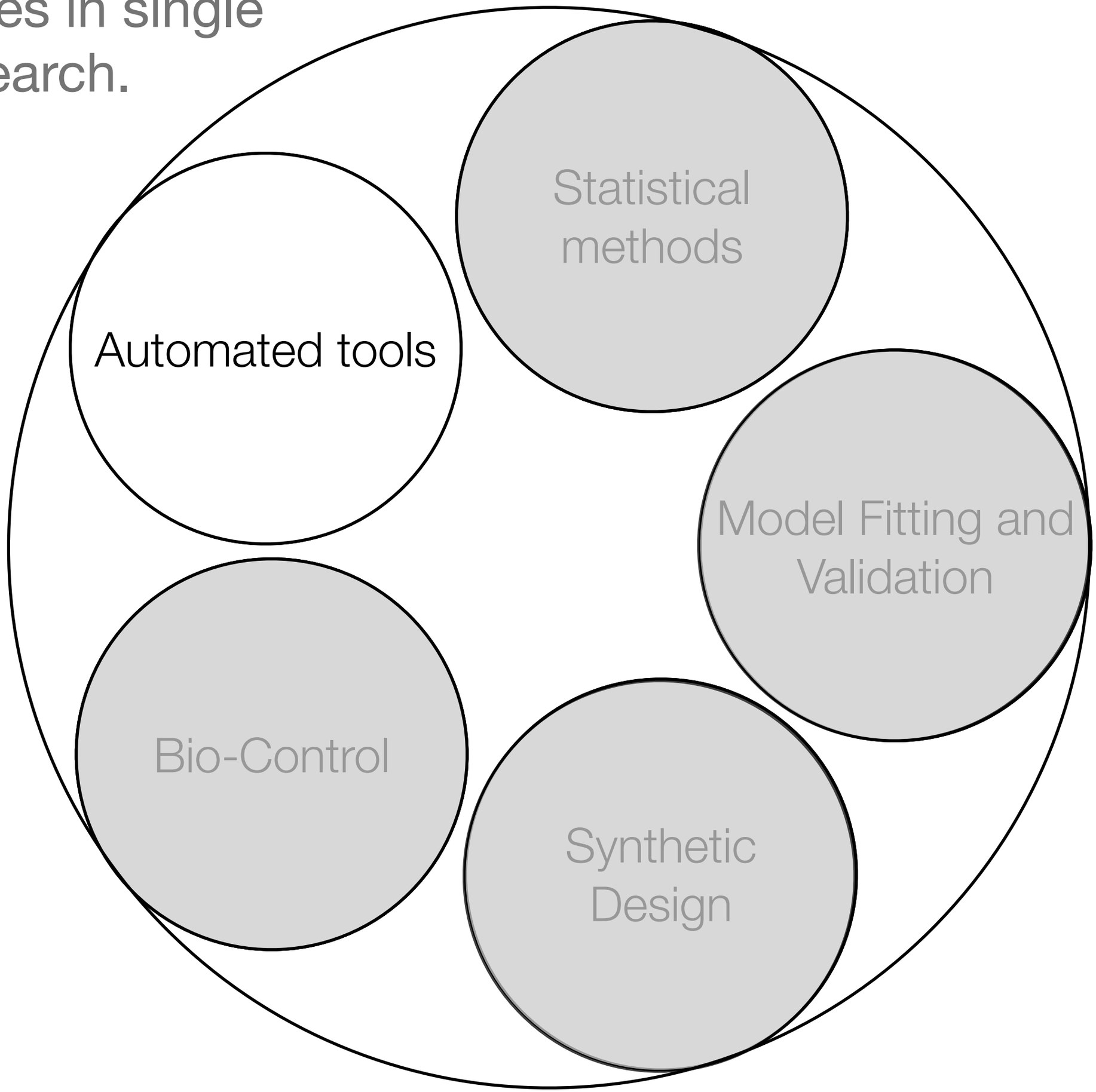

Flow Cytometry and fluorescence activated cell sorting

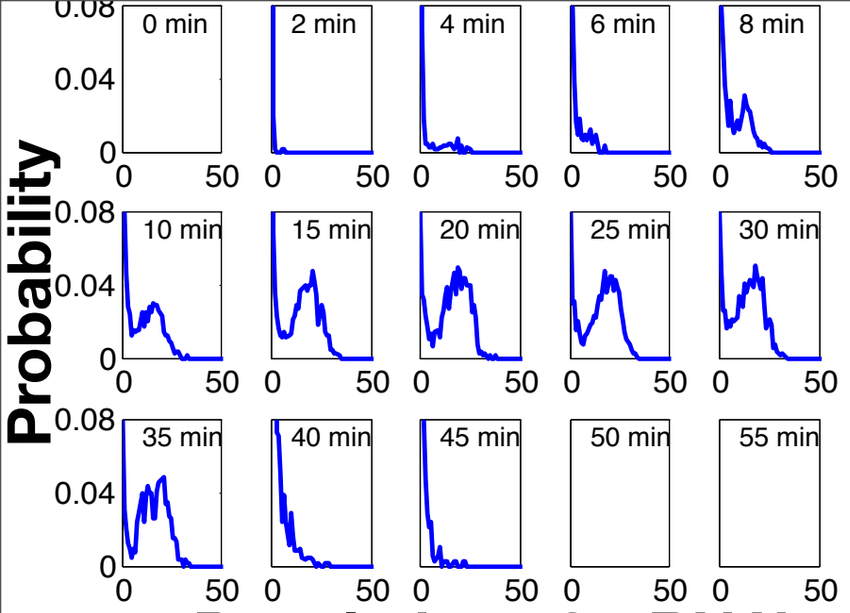


Single molecule Fluorescence *in situ* Hybridization (FISH)

Raj, Nature Methods 2007

Advances in single cell research.



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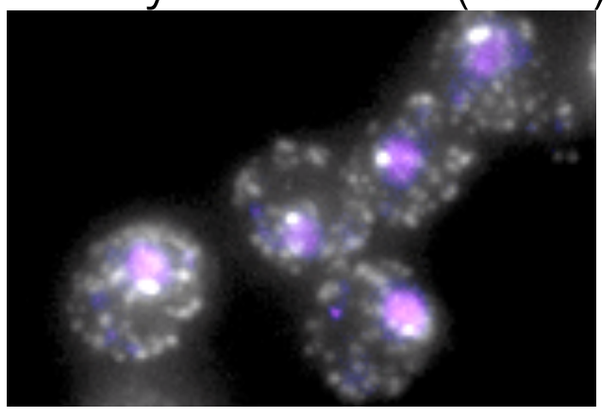
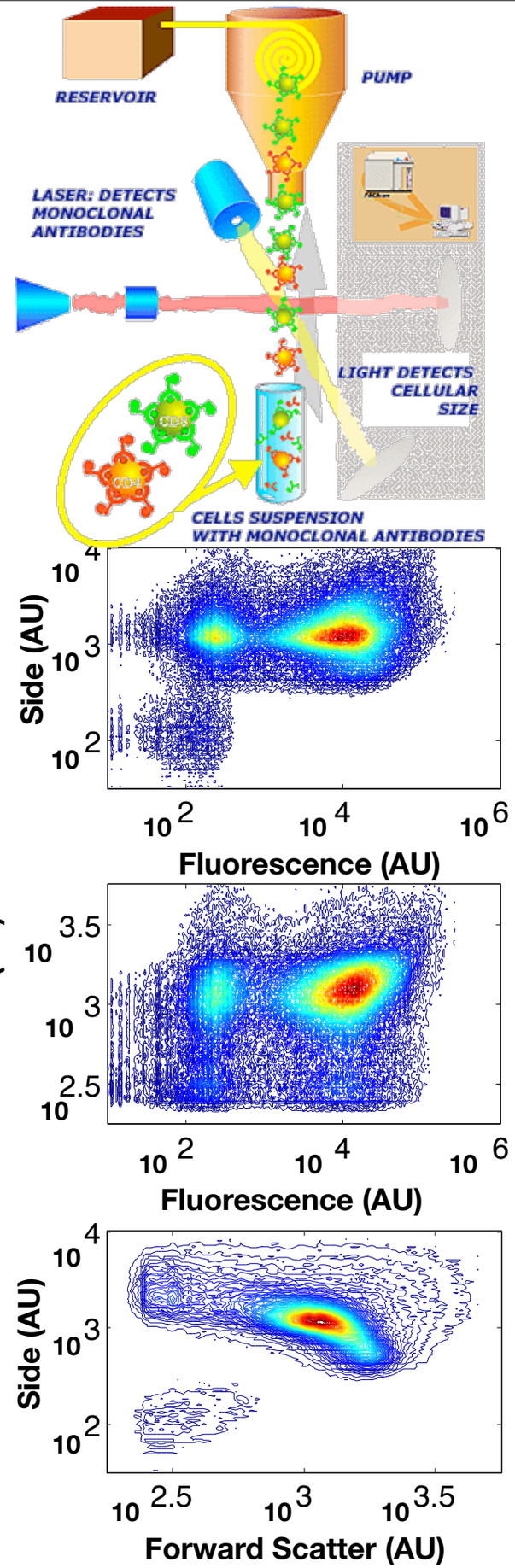
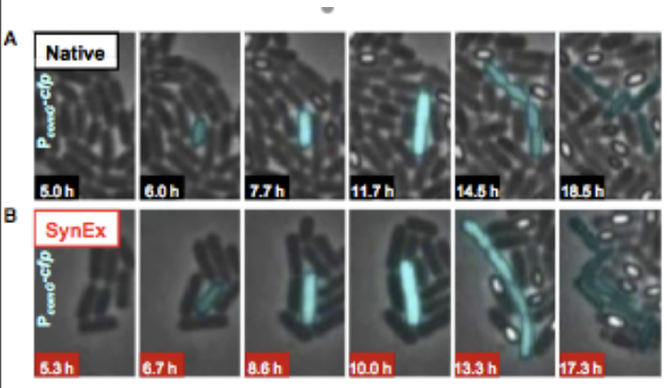
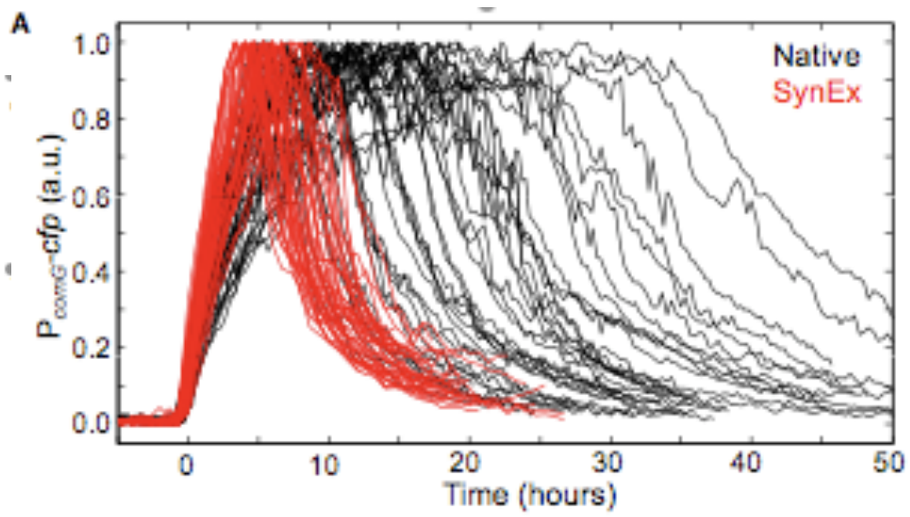


Statistical methods



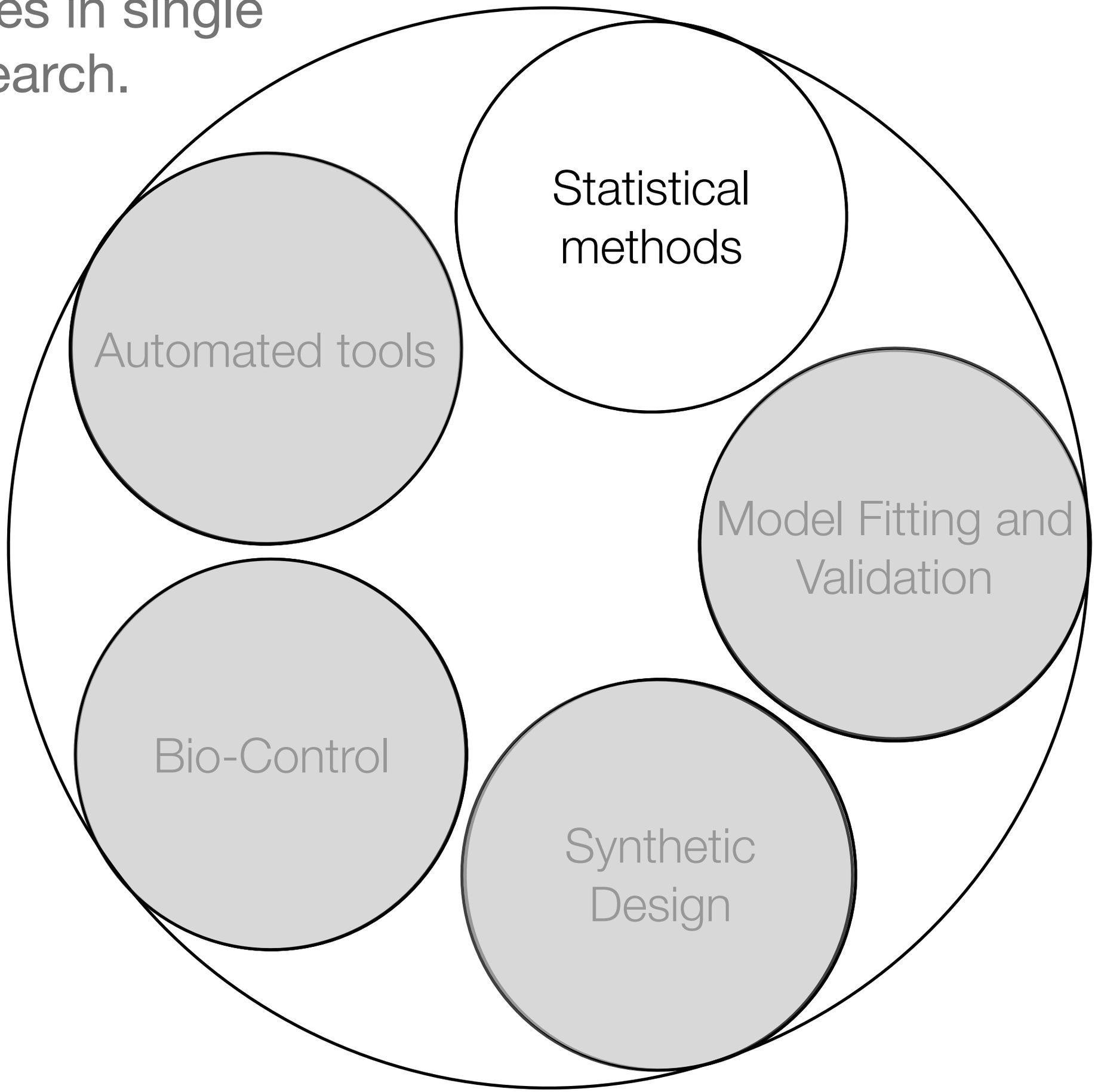

Flow Cytometry

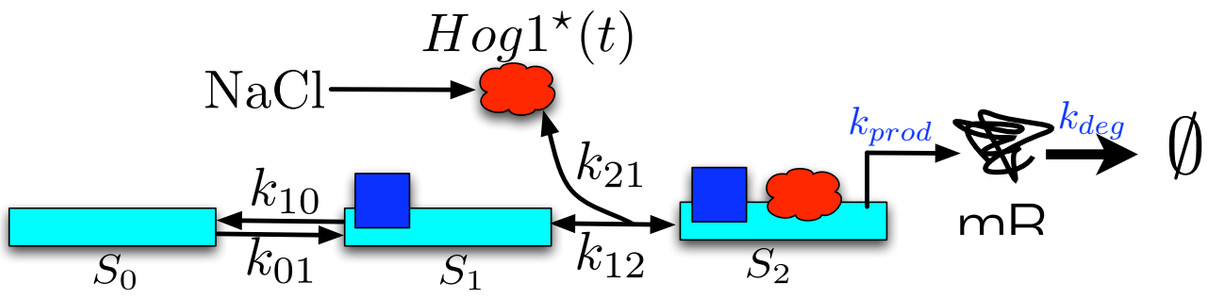


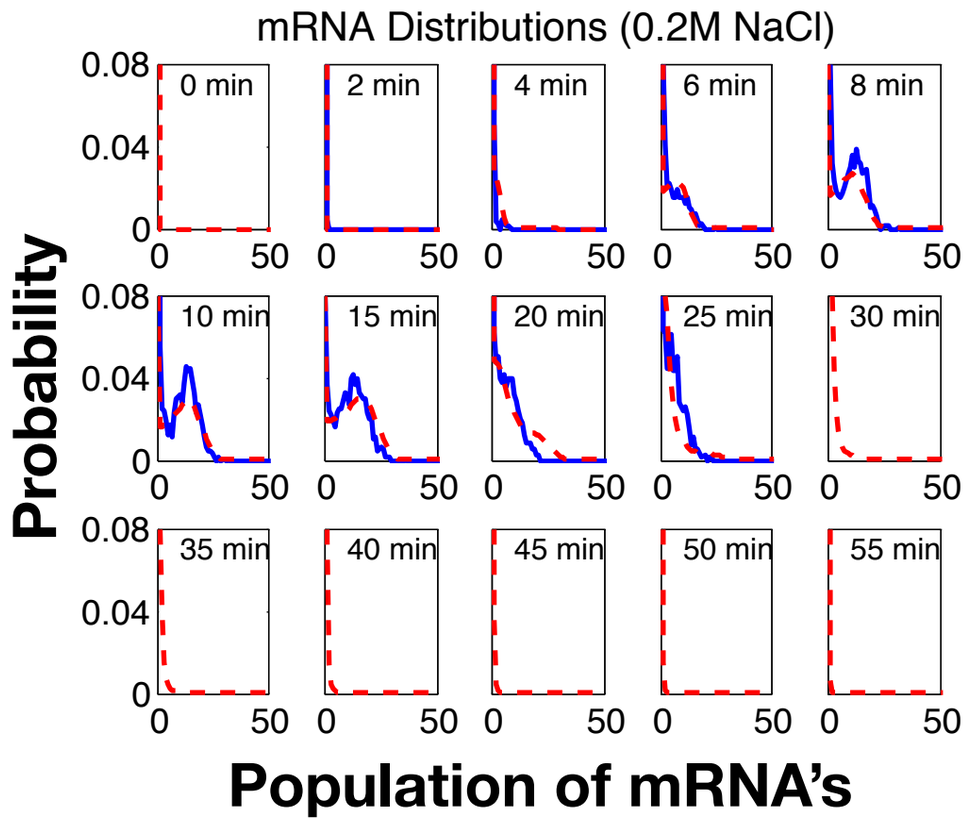




Time lapse fluorescence microscopy Cagatay et al, Cell 2009

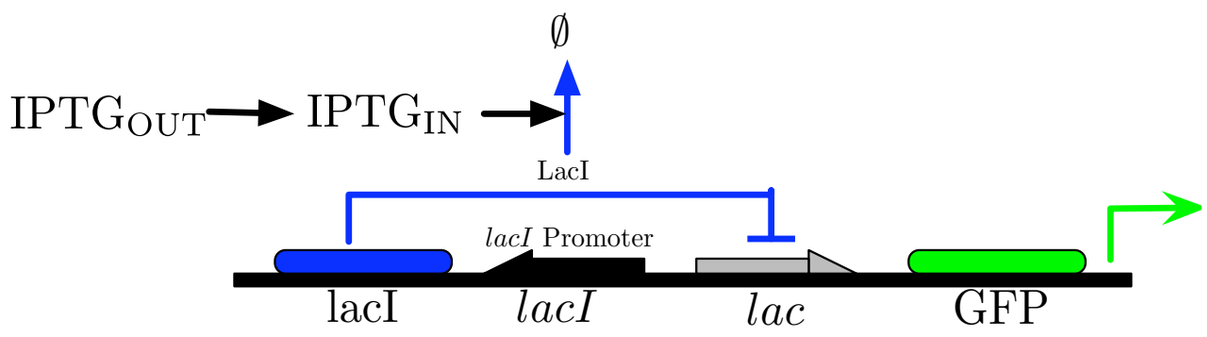
Advances in single cell research.

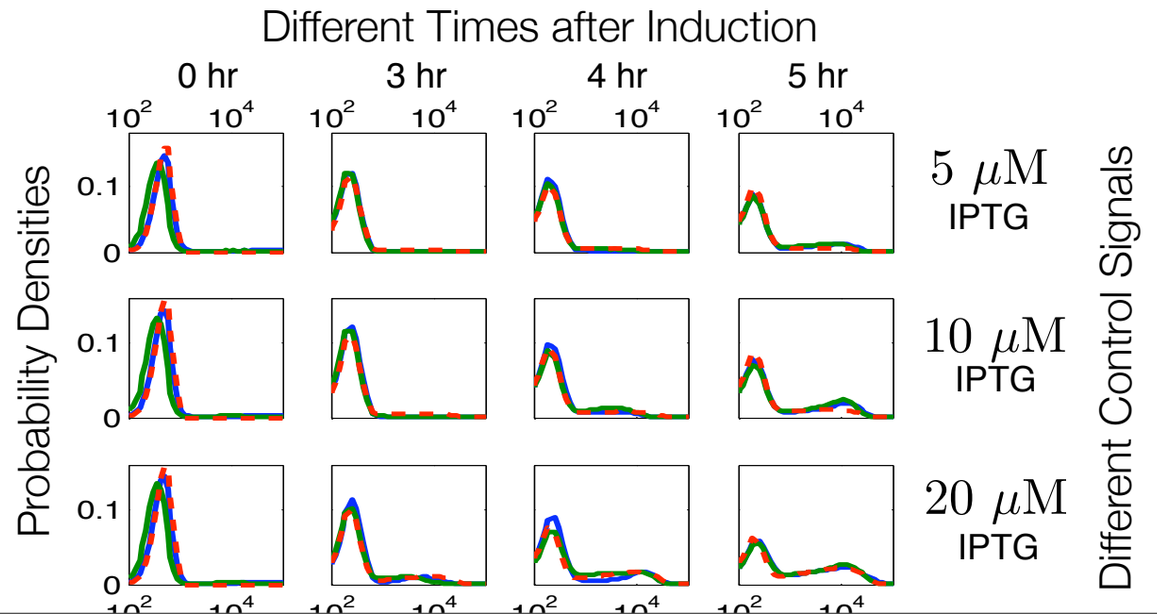




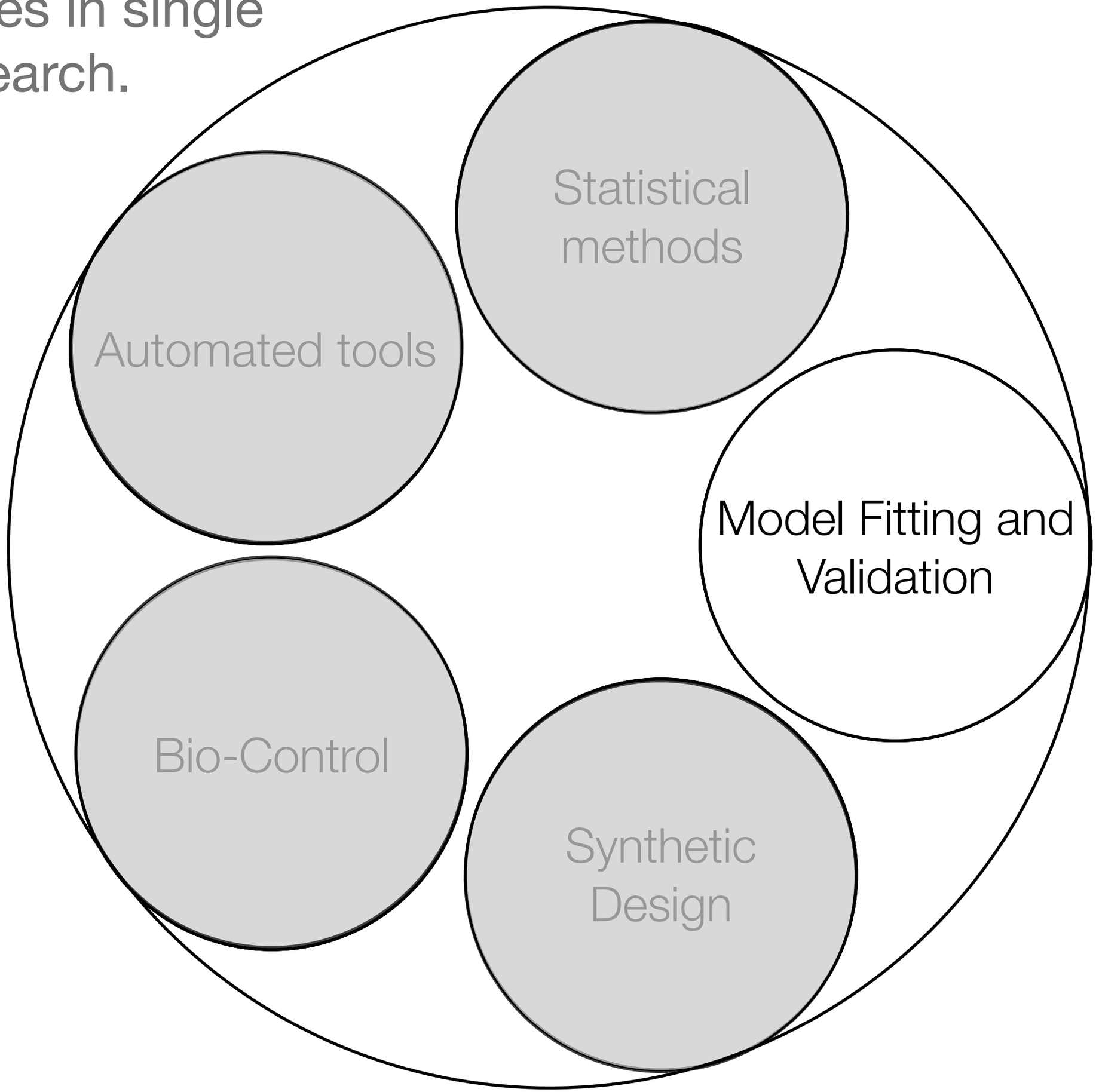



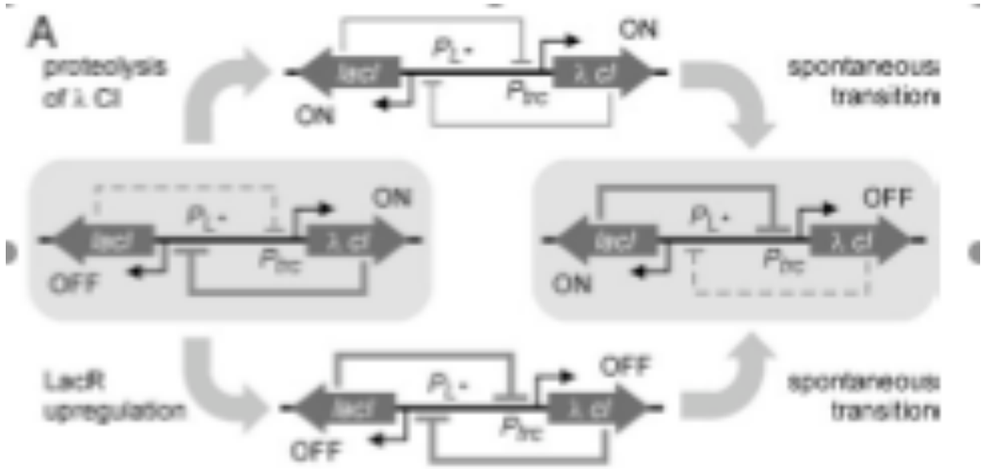

Model Fitting and Validation



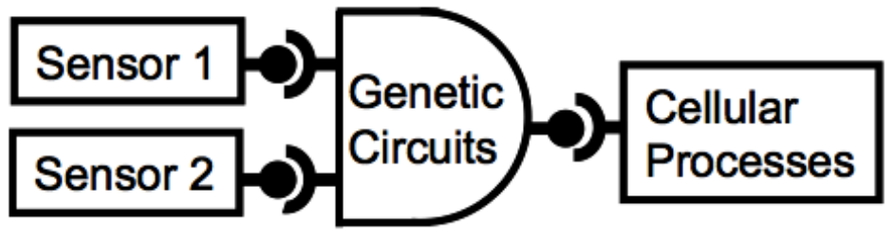



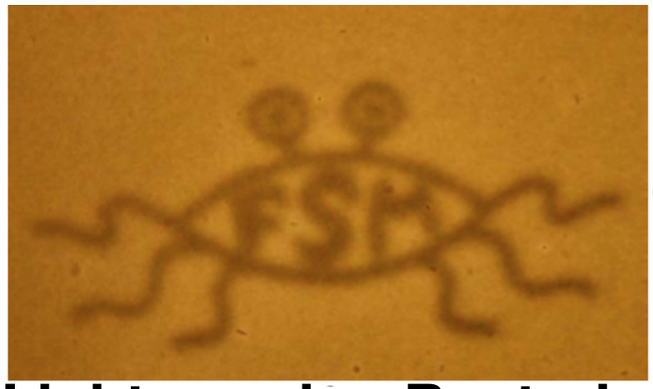
Advances in single cell research.



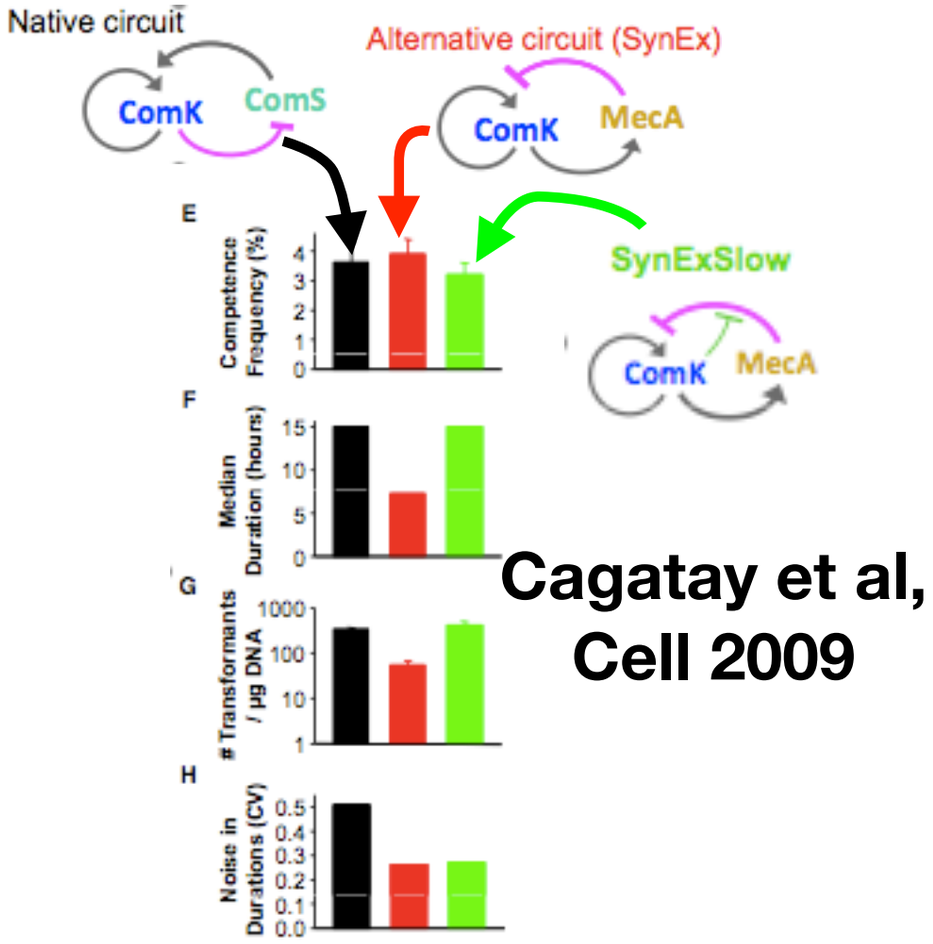



**Genetic Toggle Switch, Kobayashi *et al,* 2004**



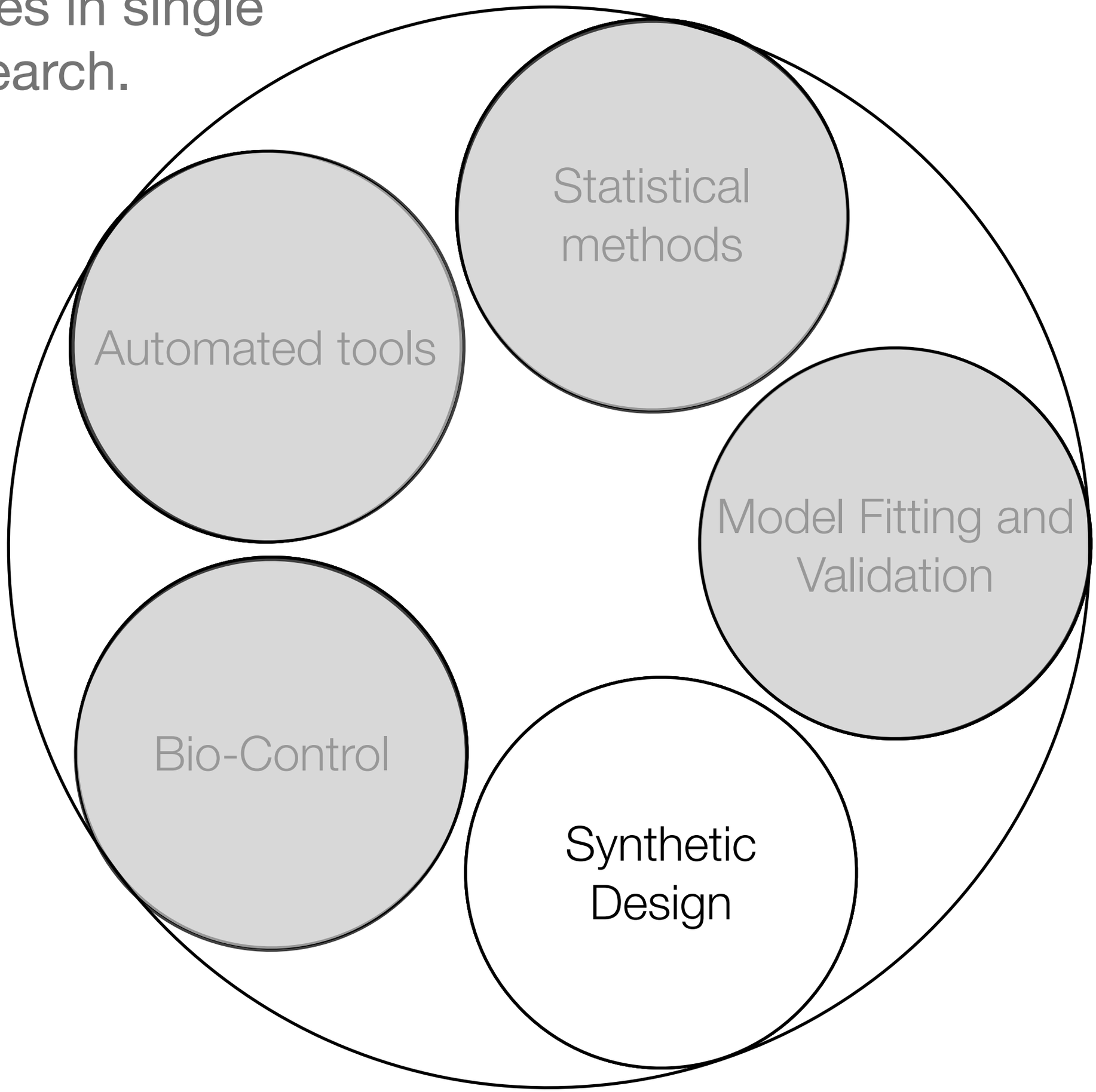
**Light sensing Bacteria, Voigt Lab, 2005**

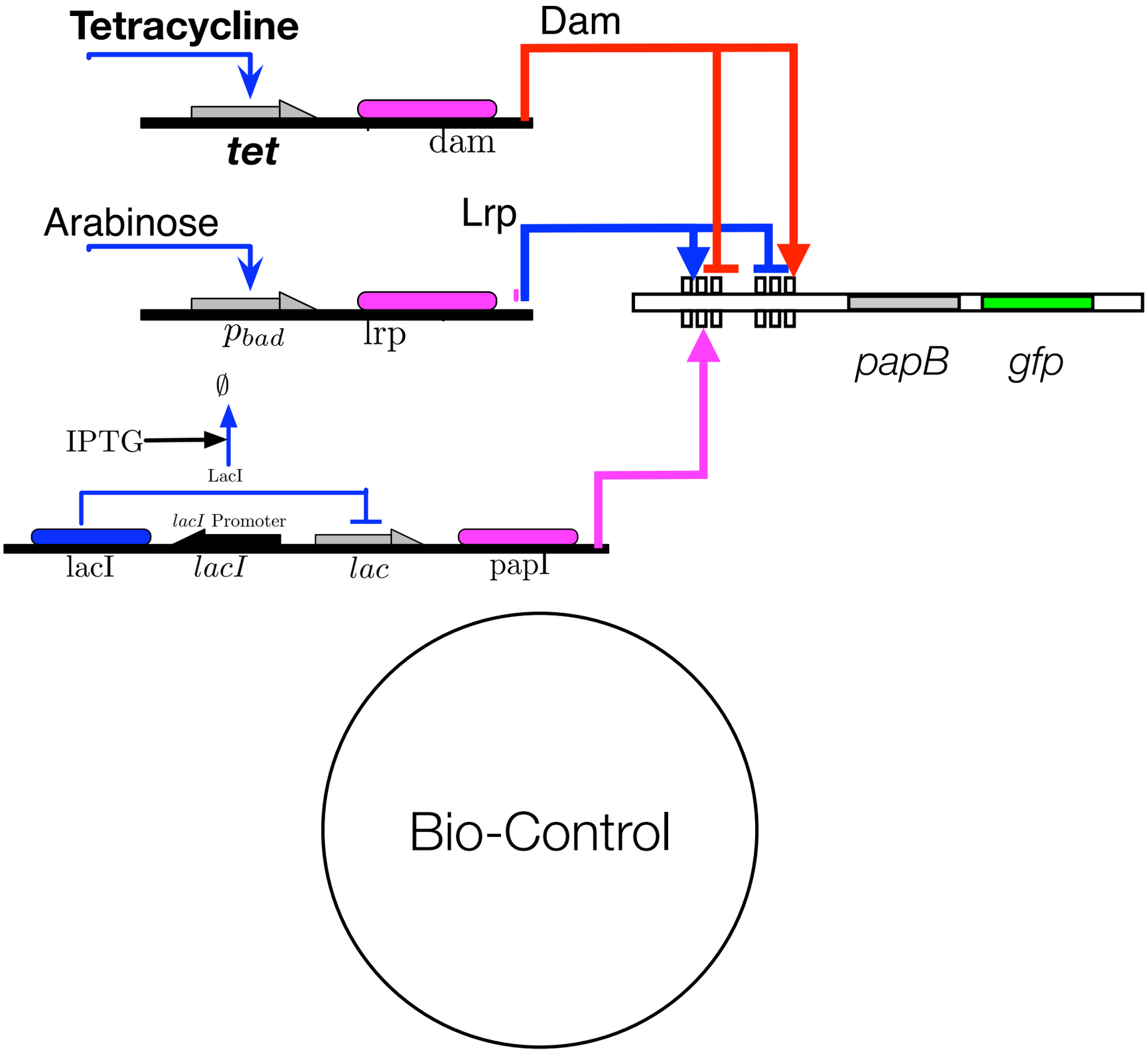



**Cagatay et al, Cell 2009**

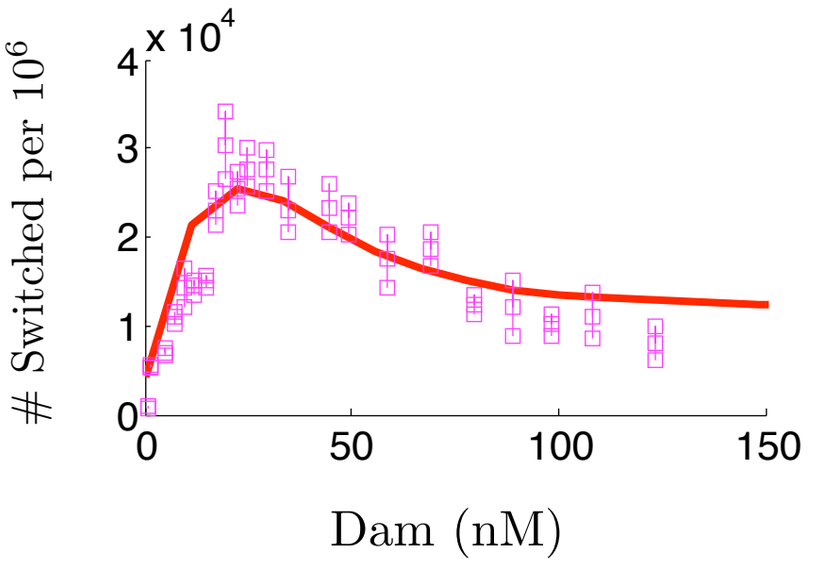
Synthetic Design

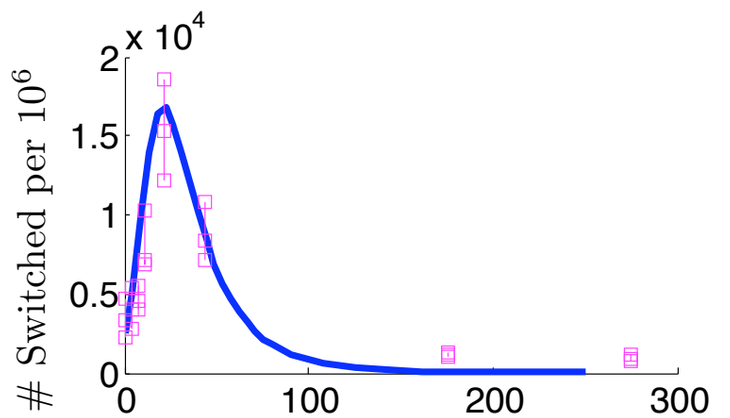
Advances in single cell research.

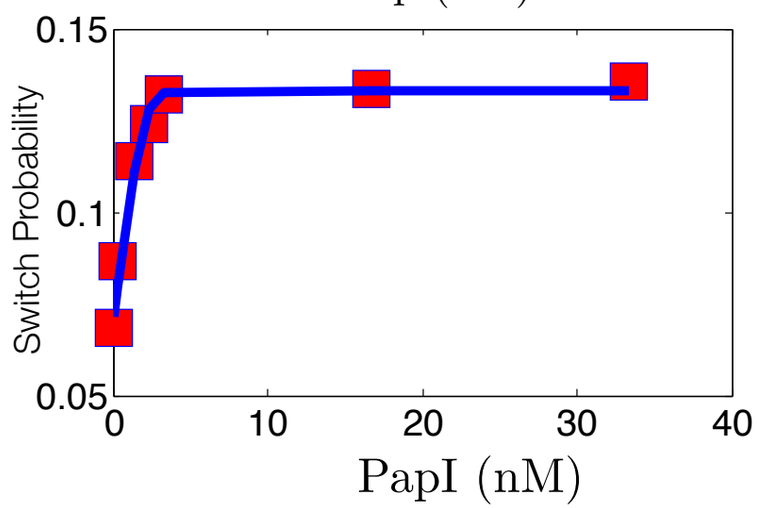



Tetracycline
Dam
tet
dam
Arabinose
Lrp
$p_{bad}$
lrp
papB
gfp
∅
IPTG
LacI
lacI Promoter
lacI
lacI
lac
papI

Bio-Control


# Switched per $10^6$
x $10^4$
4
3
2
1
0
0
50
100
150
Dam (nM)


# Switched per $10^6$
x $10^4$
2
1.5
1
0.5
0
0
100
200
300
Lrp (nM)


Switch Probability
0.15
0.1
0.05
0
10
20
30
40
PapI (nM)

# Stochastic Biochemistry: Lecture Plan

1) Theoretical Techniques
(Munsky, Synitsyn, ten Wolde)

2) Experimental Techniques
(Werner, Hong-Geller, Neuert)

# Lecture Plan:
# 1) Theoretical Techniques

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- Today and Tomorrow--Brian Munsky (LANL - CNLS)
  - Modeling of stochastic effects in systems biology.
- Friday, July 29--Nikolai Synitsyn (LANL - T4 )
  - Moment Generating Function approach to analyzing biochemical stochasticity
- Tuesday, August 9-- Pieter Ren ten Wolde (AMOLF)
  - Spatio-Temporal Correlations in Biochemical Systems
- Wednesday, August 10-- Ilya Nemenman (Emory)
  - Signal processing in biochemical networks (Tutorial session at conference)
- Wednesday, August 10-- Brian Munsky and Gregor Neuert (MIT)
  - Identifying signal-activated GRN's by integrating single cell measurements and stochastic analyses (tutorial as conference).

# Lecture Plan:
# 2) Experimental Techniques

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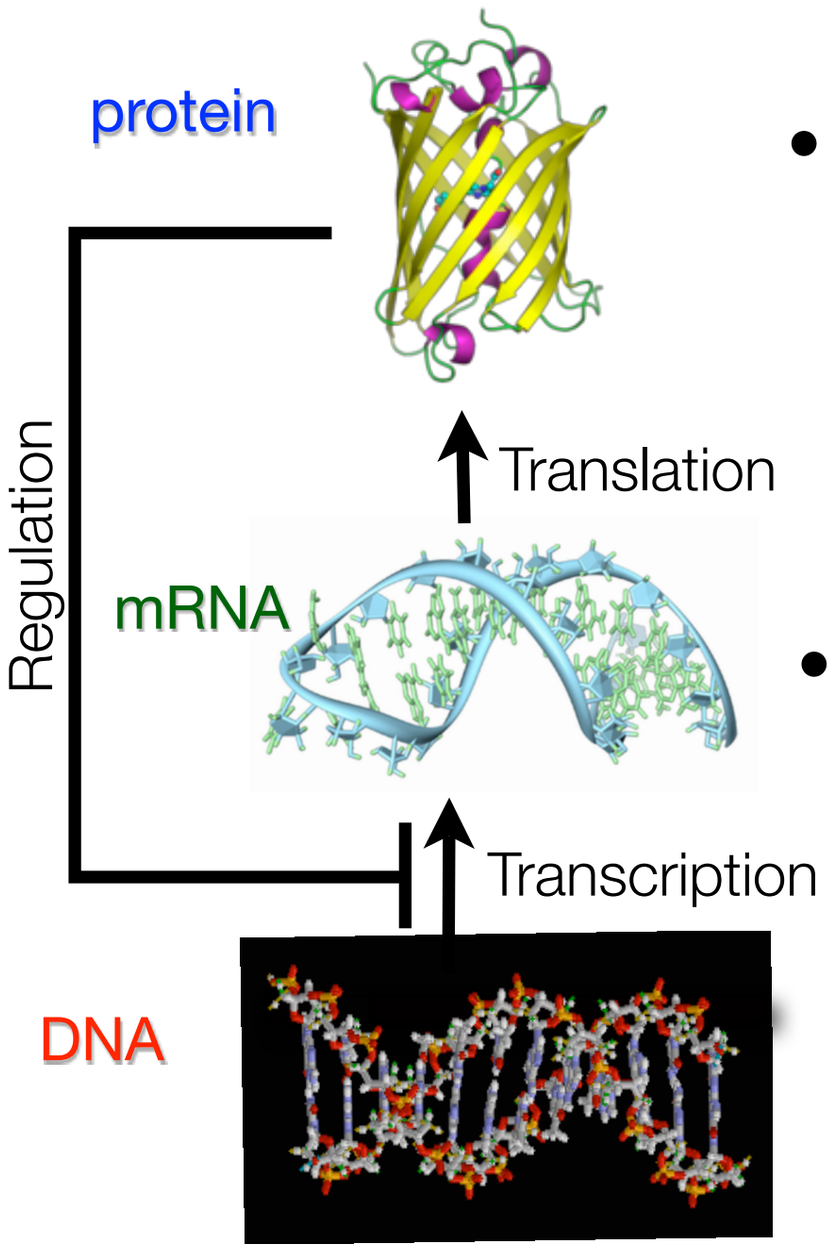
- Wednesday, August 3--Elizabeth Hong-Geller (LANL - B7)
  - Molecular tools for the analysis of Gene Regulstion
- Wednesday, August 3--Jim Werner (LANL - CINT)
  - Fluorescence Correlation Spectroscopy (FCS) and 3 Dimensional Single-Molecule Tracking
- Wednesday, August 4--Gregor Neuert (MIT)
  - Integrating single cell data and stochastic models.

# Lecture 1: Modeling of stochastic gene regulation (Part 1).

# On the menu...

- Today (Part 1)
  - Solutions for Simple Stochastic Processes (Transcription)
  - Importance of Population Size
  - Stochastic Chemical Kinetics
  - Moment Computations for Linear Propensities
  - Moment Closures for Non-Linear Propensities
- Tuesday (9:00-10:45) (Part 2)
  - Monte Carlo Simulation Techniques
    - Gillespie (SSA), Tau leaping, Chemical Langevin (SDEs), Slow Scale SSA.
  - Density Computations with Finite State Projection Techniques
  - Switch and Trajectory Analyses
  - Examples and software

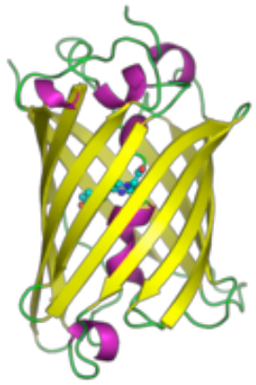
# The Central Dogma of Molecular Biology



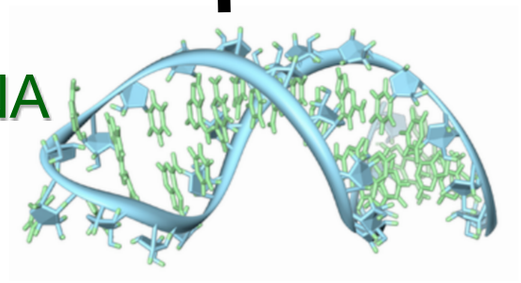

- Proteins assemble to build cellular structures, pass cellular information and regulate cellular activities.
- mRNA transfer instructions for the creation of specific proteins.
- DNA contains all of the genetic instructions.

# The Central Dogma of Molecular Biology

protein

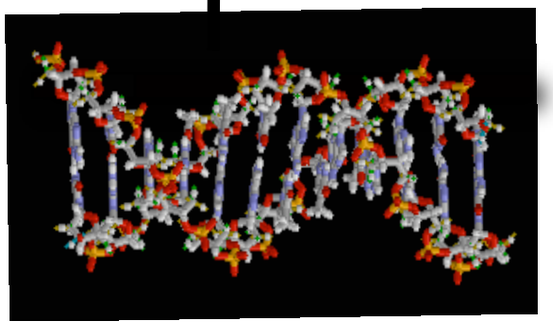


Translation



mRNA

Transcription



DNA

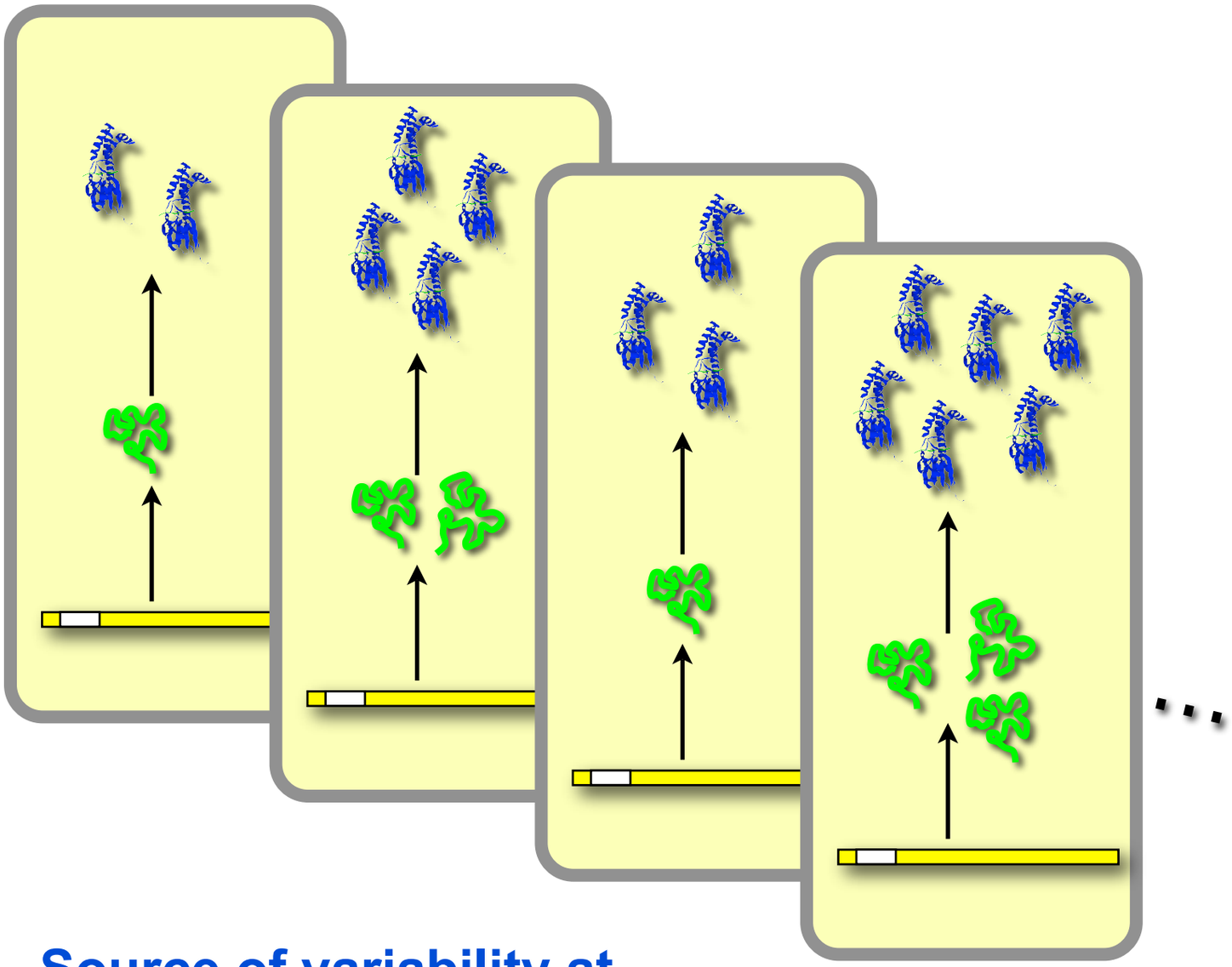
## Deterministic model

$$\frac{d[mRNA]}{dt} = -\gamma_r[mRNA] + k_r$$
$$\frac{d[protein]}{dt} = -\gamma_p[protein] + k_p[mRNA]$$

## Stochastic model

- Probability a single mRNA is transcribed in time $dt$ is $k_r dt$.
- Probability a single mRNA is degraded in time $dt$ is $(\#mRNA) \cdot \gamma_r dt$

# Intrinsic Variability in Gene Expression



**Source of variability at cellular level….**

- Small # of molecules
- Random events

*“Intrinsic noise”*

## Impact of variability

- Noise propagates through the network
- Its amount depends on
  - # of molecules
  - stoichiometry
  - regulation
  - ...
- Sometimes it is suppressed; other times it is exploited
- Deterministic models are not adequate

Slide Contributed by Mustafa Khammash